WWARN

WWARN PHARMACOLOGY MODULE

Statistical Analysis Plan

31.05.2012

Treatment Outcome - Day 7 Lumefantrine Concentration Analysis

1. Introduction

While there are probably more studies published on the pharmacokinetics of lumefantrine than any other antimalarial, available published data are not sufficient to develop optimal evidence-based dosage recommendations for all major target population groups. The "therapeutic" day 7 lumefantrine concentrations published to date range widely between 170 ng/mL to 500 ng/mL, yet many studies report a high proportion of patients with lower drug exposure despite supervised administration of the currently recommended dose. An accurate definition of the day 7 concentration (and area under the concentration time curve, AUC) that achieve an adequate clinical and parasitological response is essential for identifying target populations in whom dose optimisation studies are required. To date, lower lumefantrine exposure has been described in young children, pregnant women, bodyweight >80kg, smokers or when artemether-lumefantrine is taken unsupervised, without fat, or with mefloquine. However, the extent to which this compromises efficacy is poorly defined, and no dose optimisation studies have been conducted in these important target populations.

This pooled PK-PD data analysis is being conducted under the auspices of the Pharmacology Module of the WorldWide Antimalarial Resistance Network (WWARN). WWARN aims to ensure that anyone affected by malaria receives effective malaria treatment. WWARN will provide urgently needed, comprehensive, timely and quality-assured intelligence to track the emergence of malarial drug resistance (see www.wwarn.org). The main goals of its Pharmacology module are to improve the quality of antimalarial pharmacology data generated worldwide and to use this information to define drug resistance accurately (by distinguishing it from inadequate drug exposure) and inform optimal dosing schedules.

Researchers that have published relevant studies that have reported lumefantrine concentrations were invited to participate in this pooled analysis. The contributed data were uploaded to chassis and data curation followed the methods described in the pharmacology module Data Management and Statistical Analysis Plan vs1 (DMSAP) available online at http://www.wwarn.org/sites/default/files/PharmacologyDMSAP.pdf

2. Objective

The objective of this analysis is to define therapeutic day 7 plasma lumefantrine concentrations for the treatment of *Plasmodium falciparum* malaria and prevention of reinfection. For this purpose, pooled data from all available lumefantrine studies with clinical and pharmacological data will be used.

3. Study eligibility criteria for inclusion in the pooled analysis

Any study in malaria patients treated with artemether-lumefantrine over a 3 day period, with lumefantrine concentration measurement available on Day 7 and treatment outcome available within at least 28 days follow-up will be included in this pooled analysis. Studies (or study arms) using 5 day regimens will not be included as concentrations at day 7 after 5 day regimen have

different properties than those after 3 day regimen as they are most likely not to be at the terminal elimination phase yet.

4. Patient exclusion

The following patients will be excluded from the analysis:

i. No plasmodium falciparum infection on enrolment
   Patients with mixed species infections with P. falciparum will be included.
ii. Severe malaria at baseline or in the first 7 days
iii. Any of these other deviations, as defined by Clinical Module DMSAP :
   (a) Haemoglobin < 5 g/dL on day 0
   OR
   (b) Haematocrit < 15% on day 0

Patients with hyperparasitaemia on enrollment will be included in the analysis, provided the concentration and outcome data are collected. Patients with no recorded parasitemia on day 0 but parasitaemia recording on the following days will be also included in the analysis.

Table comparing clinical characteristics of patients included in the clinical studies and patients included in the PK analysis will be presented.

5. Definitions

Definition of Day 7

Concentration measurement will be treated as taken on Day 7 if it was recorded anytime between 144 and 196 hours, inclusive or the exact time of measurement was not recorded but the day of measurement was specified as day 6 or day 7 or day 8. The approximate time in days will be converted to time in hours by multiplying it by 24. In the analysis only one day 7 concentration per subject will be used so if there are two or more measurements within 144-196 hours the following rules will be applied:

(i) concentration were measured on both sides of 168 hours between 144 and 196 hours -

linear regression will be fitted to log-transformed concentrations closest to the 168h (one on each side) to estimate the concentration at 168 h.

(ii) concentrations were measured between 144 and 196 hours on the same side of 168h -

the measurement which is closest to 168 hours will be selected.

Concentrations below level of quantification will be replaced by half of the limit of quantification and used in these calculations.

Treatment Outcome

WWARN Outcome and Study Outcome

WWARN Outcome is generated, as prescribed in Clinical Module DMSAP http://www.wwarn.org/sites/default/files/ClinicalDMSAP.pdf , based on weekly recordings of parasitaemia between day 7 and the last day of follow-up and PCR results. Gap in parasitaemia recordings of 18 days or longer is considered as a deviation and patients with such a gap are treated as if they were lost to follow-up at the last visit before the gap.

For each PK study an outcome variable defined by the study investigators is also available ("PI outcome"). In some patients this outcome maybe be different from WWARN outcome, for example due to the missing parasite count data. In these situations the following rules will be applied:

(i) Patients withdrawn from the study due to AE reported who have completed follow-up visits will be discussed with investigator in order to be included.
(ii) PI outcome = recurrence ; parasitaemia missing for the day of recurrence but PCR result is available - accept PI outcome
(iii) PI outcome = recurrence; parasitaemia missing for the day of recurrence and PCR not available - accept WWARN outcome (i.e. lost to follow up)
(iv) PI outcome = recurrence; parasitaemia=0 recorded on the day of recurrence check with PI
(v) PI outcome= ACPR, ≥18 days gap – censor before the gap (i.e. WWARN outcome)
(vi) PI outcome= recurrence; ≥18 days gap - outcome according to recorded parasitaemia and PCR after the gap

All patients with outcome beyond day 63 will be censored at day 63.

Definition of transmission intensity

Transmission intensity should be defined based on geographical location and calendar month of the study. Observed rate of new infection may be a more accurate measure of transmission intensity then the classification given in the publication which most often refers to the data from the past. However, other possible factors affecting re-infection rate within study, such use of bednets and post treatment prophylactic effect of long acting antimalarials need to be considered.

Therefore, transmission intensity will be defined based on triangulation of information given in the publication(s) (location, type of malaria transmission, use of bed nets, month of study), observed reinfection rate and expert opinion through the discussion within the study group. Transmission intensity will be classified as low, moderate or high.

6. Efficacy Endpoints

Three separate analyses will be performed for the following endpoints:

Plasmodium falciparum recrudescence

For the analysis of Plasmodium falciparum recrudescence, failure will be defined as PCR confirmed Pf recrudescence. Patients who had a new infection (of any species) will be censored at the first day

the positive parasitaemia was recorded. Patients who had a reappearance of Pf parasites but the PCR result is not available will be excluded from the analysis to avoid informative censoring. Patients with recurrent parasitaemia have higher risk of recrudescence then patients in the whole population who were lost to follow-up.

Plasmodium falciparum re-infection

For the analysis of Plasmodium falciparum re-infection, failure will be defined as PCR confirmed Pf re-infection. Patients who had a new infection other than Pf species or Pf recrudescence will be censored at the first day the positive parasitaemia was recorded. Patients who had a reappearance of Pf parasites but the PCR result is not available will be excluded from the analysis to avoid informative censoring. Patients with recurrent parasitaemia have higher risk of recrudescence then patients in the whole population who were lost to follow-up.

Plasmodium vivax re-infection

For the analysis of Plasmodium vivax re-infection, failure will be defined as re-infection with Plasmodium vivax. Patients who had a new infection other than Pv species will be censored at the first day the positive parasitaemia was recorded (tehreofre patients with Pf recurrent parasiteamia but no PCR resulst will be included in the analysis and censored). Only patients from areas which are affected by Plasmodium vivax will be included in the analysis, i.e South-East Asia.

7. Covariates analysis

Based on variables which were collected across studies the following variables will be examined for their association with treatment outcome or concentration on Day 7:

Recrudescence: lumefantrine concentration on Day 7, time of sampling, initial parasitaemia (after log-transformation); parasitaemia on Day 1, 2, 3 (after log-transformation, or binary variable: positive/negative); PRR in the first 48 hours; temperature; anaemia; haematocrit/haemoglobin; gametocytes carriage on admission; patient age (within transmission intensity); malnutrition status in children, transmission intensity; year of study; drug manufacturer; matrix.

New infection: lumefantrine concentration on Day 7, time of sampling, patient age, malnutrition status in children, transmission intensity, year of study, drug manufacturer.

Plasmodium Vivax infection: lumefantrine concentration on Day 7, time of sampling ,patient age; transmission intensity, malnutrition status in children, year of study; drug manufacturer

Concentration on Day 7: mg/kg dose given, treatment administration with fat; supervised treatment administration (yes,no, partially); drug manufacturer; patient age; ethnic origin (country); drug manufacturer; malnutrition status in children; sample matrix; haematocrit/haemoglobin; initial parasitaemia, time of sampling post dose; HIV coinfection, concomitant medication.

Effect of age will be examined within transmission intensity, i.e. interaction between these two variables will be examined. Age will be considered as a continuous variable and also as categorical with 4 categories: <1 year; 1-4 years, 5-11 years, >=12 years.

Parasitaemia on Day 1, 2 and 3 and PPR at 48 hours will be considered as covariates for the recrudescence analysis as it could be a more relevant measure of parasite load left for the lumefantrine to eliminate than the initial parasitaemia since it accounts for the initial substantial decrease in parasitaemia due to the artemether administration.

Slow early parasitological response will be defined based on PRR at 48 hours (looking at the distribution for all patients) and parasite positivity on Day 3 and also be evaluated as a covariate.

Anaemia will be defined according to WHO guidelines http://www.who.int/vmnis/indicators/haemoglobin.pdf . For studies where only haemoglobin was measured, the following formula will be used to estimate haematocrit: Haematocrit = 5.62 + 2.60 × Haemoglobin (Lee et al, 2008).

Nutritional status of children aged <5 years will be assessed as data permit using the igrowup package developed by WHO (2006).

8. Statistical Methods

Summaries and Visualisation

Summary tables of available data by study will be presented giving details of:

1. study population:
   publication reference; country; year; regimen; arm, age; number of subjects in population (total, pregnant, ≤1 year, 1-4 years, ≥5 years, HIV test, HIV status);
2. dosing:
   N with full dosing data (actual dose and dosing times for each dose and observed body weight recorded); N with dosing data but not for all doses; N with missing individual dosing information, N with missing weight; time since first dose for Dose 2 – Dose 6, Total Dose (mg/kg).
3. PK sampling for drug:
   matrix, Lower limit of quantification (LLOQ), protocol sampling times, total number of concentrations below LLOQ; median (range) of number of concentrations per subject; total concentrations with sampling time data (exact, protocol time in hours, protocol time in days); N with Day 7 data; N with AUC after last dose.
4. PK sampling for metabolite:
   matrix, LLOQ, protocol sampling times, total number of concentrations below LLOQ; median (range) of number of concentrations per subject; total concentrations with sampling time data (exact, protocol time in hours, protocol time in days).
5. outcome:

duration FU;
number of patients with: ACPR, LTFU, recurrence;
PCR results: recrudescence, new P falciparum infection, P vivax, other species, indeterminate, missing

6. demographics:
   sex; age; weight; height; body mass index
7. baseline characterisctics:
   laboratory measures at baseline
   symptoms at baseline
8. weekly gametocytes counts on days 0 to 42

Also, table summarising demographics and most common baseline data for all studies combined (median (range); N(%) of patients with non-missing value, N(%) of records (concentrations) with non-missing values) will be presented, separately for pregnant and non-pregnant patients.

All measured concentrations will be plotted for each study, showing different sampling matrices used. Distribution of measured concentrations on Day 7 will be presented overall and for each transmission intensity showing different sampling matrices, and will be presented separately for pregnant and non-pregnant women.

For the purpose of plots listed below, outcome at time x will be defined as a binary variable in the following way:

Reinfection = 1 if reinfection occurred before or at time x; 0 if patient reached time x and no reinfection or recurrence was observed until x. All patients with day of last outcome before day x and outcome other than reinfection will be excluded.

Recrudescence= 1 if recrudescence occurred before or at time x; 0 if patient reached time x and no reinfection or recurrence was observed until x. All patients with day of last outcome before day x and outcome other than recrudescence will be excluded.

Separate plots will be presented for reinfection and recrudescence.

Relationship between lumefantrine concentration on Day 7 and outcome (ACPR vs. recrudescence or reinfection) will be presented visually in the following plots for outcomes defined at 28 and 42 days, separately for pregnant /non-pregnant patients:

1. Plot of day 7 concentration versus initial parasitaemia, observations marked by outcome and matrix (different colour and shape of markers will be used); overall and by transmission intensity
2. Box plot of day 7 concentrations on day 7 by outcome , overall and by transmission intensity, and by age within each transmission intensity showing concentrations separately for each matrix

The following plots exploring relationship between day 7 concentration and dose will be also presented:

3. Plot of day 7 concentration versus weight adjusted total dose given; observations marked by outcome and sampling matrix (different colour and shape of markers will be used); overall and by transmission intensity, and by age within each transmission intensity.

Regression analysis of Day 7 concentration

Factors affecting concentration on Day 7 will be examined using a linear regression model with random effects for study-site to account for heterogeneity between studies. Concentration will be log-transformed as it is log-normally distributed. Residuals will be examined to assure they are normally distributed.

Inclusion of covariates in the final model will be determined based on how they improve the overall model (likelihood ratio test) and if they change the coefficient estimates for other factors and based on the residuals.

This analysis will be also be used to explore relationship between day 7 concentrations measured using different matrices.

Since a variable coding supervised treatment administration does not describe individual patient dosing history adequately, subset analysis will be performed only on patients who received supervised treatment to avoid possible confounding with other covariates.

Regression analysis of treatment outcome

Survival regression analysis for risk of recrudescence and, separately, for risk of reinfection, will be performed. Random effects in the form of frailty parameters will be used to adjust for study-site effect (Glidden and Vittinghoff, 2004). Separate models will be fitted for time to recrudescence and time to new infection as these events have different risk functions over time and possibly different risk factors. Patients with no PCR results (missing or indeterminate) will be excluded from both analyses due to informative censoring. Patients with recurrent parasitaemia have higher risk of recrudescence / reinfection then patients in the whole population who were lost to follow-up.

As the main objective of these analyses is to understand relationship between Lumefantrine Day 7 concentration and treatment outcome, and the effect of lumefantrine will cease with time (as the concentration will decrease), the follow-up time will be truncated to 21, 28, 35 and 42 days and regression analysis will be performed to find a period of time during which there is a strongest association between lumefantrine concentration at day 7 and outcome. Likelihood ratio statistics will be used to assess the association between outcome and concentration, but models will also be selected based on overall goodness of fit.

A Cox regression model and models with parametric hazard functions such as: Gompertz, Weibull, lognormal and log-logistic will be examined and the best regression model will be selected based on Cox-Snell residuals (Collett, 2003). In the Cox regression model, the proportional hazard assumption will be tested based on Schoenfeld residuals (Schoenfeld, 1982).

Inclusion of covariates in the final model will be determined based on how they improve the overall model (likelihood ratio test) and if they change the coefficient estimates for other factors and based on the residuals. Variables will be examined for inclusion in the final model in a stepwise forward fashion.

A final regression model will be used to estimate probability of treatment success at time x for each patient. The relationship between this probability and the concentration on day 7 will be examined and used to determine therapeutic concentrations for recurrence and new infection.

Sensitivity analysis

All final models will be refitted with one study excluded at a time and estimates of coefficients for all parameters will be noted and summarised. This will identify influential studies, i.e. studies which are different from the others and affect the overall estimates.

Sensitivity analysis will be performed on the final models for recrudescence and new infection with respect to:

1. Timing of Day 7 sampling
2. Outcome after 18-day gap
3. Patients with no PCR

using the multiple imputation method (Little and Rubin, 2002). In summary, missing data (i.e. exact time of concentration sampling, true outcome) will be first filled in by several sets of plausible values to create multiple completed datasets, then the final model will be fitted to each of these datasets, and the covariates estimates will combined to yield a single inference.

Since we allow concentrations taken within 6-8 days window, timing of Day 7 sample is only relevant in a model with the actual time of sampling included as a covariate. Therefore, if the sampling time variable is not selected for the final model, the sensitivity analysis will be based on the final mode with sampling time variable added.

"Optimal" concentration cutoff

The relationship between lumefantrine concentrations on Day 7 and treatment response will be examined with the aim of defining optimal therapeutic thresholds, i.e the concentration that best separates those with a low and high risk of treatment failure (PCR adjusted recrudescence, PCR adjusted reinfection, PCR unadjusted recurrence of Pf parasitaemia). The optimal thresholds will be selected on the basis of logrank statistics using maximum statistic approach, with P-values adjusted using the method proposed by Contal and O'Quigley (1999).

This analysis will be performed on the whole data set and within each transmission intensity.

References

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